Supplementary material

Supplementary Methods

List of participating sites and investigators

University of Goettingen, Goettingen, Germany (Mareike Lankeit, Kristian Hellenkamp)

Medical University of Warsaw, Warsaw, Poland (Anna Wyzgał, Michał Ciurzyński, Maciej Kostrubiec, Piotr Pruszczyk)

Ramón y Cajal Hospital, Alcalá de Henares University, Madrid, Spain (David Jiménez, Vicente Gomez, Deisy Barrios, Raquel Morillo)

Txagorritzu Hospital, Vitoria, Spain (Jose Luis Lobo)

Virgen del Rocio Hospital, Sevilla, Spain (Remedios Otero)

Galdakao Hospital, Bilbao, Spain (Mikel Oribe)

Complejo Hospitalario San Millan y San Pedro, La Rioja, Spain (Manuel Barron)

La Fe Hospital, Valencia, Spain (Dolores Nauffal)

Hospital Sierrallana, Cantabria, Spain (Reina Valle)

Hospital Central Asturias, Oviedo, Spain (Celso Alvarez)

Corporacion Sanitaria Parc Tauli, Barcelona, Spain (Carmen Navarro)

Hospital San Juan de Dios, Sevilla, Spain (Consolacion Rodriguez)

Definitions

Although study protocols did not influence treatment decisions (made by the attending physicians) at any time, performance of transthoracic echocardiography (TTE) within 48 hours was strongly recommended. RV dysfunction on TTE was defined as dilatation of the RV (defined as right/left

ventricle diameter ratio >1.0 from the apical view) and / or a reduced RV function (defined as tricuspid annular plane systolic excursion, tricuspid annular plane systolic excursion (TAPSE) <16 mm). However, in 394 (46.7%) patients who have been included in a previously published study [1], RV dysfunction on TTE was defined as dilatation of the RV (defined as end-diastolic diameter >30 mm from the parasternal view, or a right/left ventricle diameter ratio >1.0 from the subcostal or apical view), combined with right atrial hypertension (absence of the inspiratory collapse of the inferior vena cava) in the absence of left ventricular or mitral valve disease. RV dysfunction on multidetector computed tomography (MDCT) was defined as RV/LV-ratio >1.0 [2, 3].

Tachycardia was defined as heart rate of ≥100 beats per minute (bpm), mild hypotension as systolic blood pressure between 90 and 100 mm Hg and hypoxia as oxygen saturation <90% (regardless whether oxygen was given or not). Renal insufficiency was defined as glomerular filtration rate (GFR) <60 ml/min/1.73 m².

Laboratory biomarker testing

Plasma biomarker levels were measured in batches after a single thaw by the Department of Clinical Chemistry of the University Medical Centre Goettingen, Germany, using commercially available assays. For measurement of copeptin, an automated immunofluorescent assay (B.R.A.H.M.S Copeptin us KRYPTOR assay carried out on the KRYPTOR compact PLUS instrument, BRAHMS GmbH, Hennigsdorf/Berlin, Germany) was used. As described in detail elsewhere [1, 4, 5], the assay is based on the measurement of the signal emitted by non-radiative energy transfer from a donor to an acceptor with time-delay (Time-Resolved Amplified Cryptate Emission, TRACE technology). High-sensitivity troponin T (hsTnT) and N-terminal pro-brain natriuretic peptide (NT-proBNP) were measured using quantitative electrochemiluminescence immunoassays (Elecsys 2010; Roche Diagnostics, Mannheim, Germany).

Supplementary Results

Calculated optimal copeptin cut-off value

The patient-cohort optimal copeptin cut-off value determined by Youden's index quantification in this study cohort was 28 pmol/l and thus only slightly higher than the predefined cut-off value of 24 pmol/l. If this patient-cohort optimised cut-off value was used instead the predefined cut-off value, the ORs for the prediction of an adverse outcome (OR, 8.3 [3.4-20.5]; p<0.001) and PE-related death (OR, 10.0 [3.0-33.5]; p<0.001) were even higher compared to the predefined cut-off value (**table 3** of the main manuscript).

Prognostic performance of the Bova score

Of 602 (71.4%) patients classified as low risk (≤2 points) by the Bova score, as many as 10 (1.7; 95% CI, 0.8-3.0%) had an adverse outcome. The rate of an adverse outcome was 4.2 (95% CI, 1.7-8.4) % in 167 (19.8%) patients classified as intermediate-low (3 or 4 points) and 5.4 (95% CI, 1.5-13.3) % in 74 (8.8%) patients in the intermediate-high risk group (>4 points). The OR with regard to the prediction of an adverse outcome and PE-related death for patients classified as intermediate-high risk according to Bova score (if tested versus all other patients) was 2.5 (95% CI, 0.8-7.7; p=0.104) and 2.1 (95% CI, 0.5-9.8; p=0.342), respectively.

An alternative approach for risk assessment – using copeptin instead of imaging modalities in the 2014 ESC algorithm

Since imaging modalities such as transthoracic echocardiography (TTE) and multidetector computed tomography (MDCT) for the assessment of right ventricular (RV) (dys-)function in acute PE are characterised by a number of limitations, we tested whether replacement of information from imaging modalities by the measurement of copeptin in the 2014 ESC algorithm can provide

comparable prognostic information. As shown in the **figure 1s**, 137 (16.3%) patients were classified as intermediate-high risk; of those, 12 (8.8; 95% CI, 4.6-14.8 %) patients had an adverse outcome and 7 (5.1; 95% CI, 2.1-10.2 %) died of PE.

Pooled data from the derivation and the validation study

To allow for an analysis of a larger number of patients and outcomes, the patient cohorts of the derivation [1] and the present validation study were pooled. The baseline characteristics and outcomes of the pooled cohort encompassing 1111 normotensive patients with PE are shown in **table 1s**. In comparison, patients of the validation cohort (included at 12 different sites encompassing non-university hospitals) had less often signs of haemodynamic compromise (mild hypotension, hypoxia and signs of RV dysfunction) compared patients of the derivation cohort (included at a tertiary referral university hospital only). Accordingly, copeptin plasma concentration were higher in the derivation cohort (13.7 [IQR, 5.9-44.5]) compared to the validation cohort (9.6 [IQR, 5.2-21.1]; p<0.001). Although, and despite a lower rate of adverse outcomes in the validation cohort (2.5; 95% CI, 1.5-3.8 %) compared to the derivation cohort (5.6; 95% CI, 3.2-9.1 %; p=0.017), elevation of copeptin ≥24 pmol/l was associated with an increased risk of an adverse 30-day outcome in the derivation [1], validation and pooled cohort (**table 2s**). Interestingly, while the step-wise biomarker-based strategy (based on hsTnT and NT-proBNP in the first step, followed by copeptin in patients with elevation of both, hsTnT and NT-proBNP) was associated with a lower Odds ratio in the present validation study and thus in the pooled cohort compared to the derivation study, the 2014 ESC algorithm plus copeptin measurement in intermediate-high risk patients was associated with a 11.1-fold increased risk for an adverse outcome in the derivation cohort and a 8.9-fold increased risk in the pooled cohort, which was higher than in the derivation study (**table 2s**). Overall, in the pooled cohort, 127 (11.4%) patients were identified as being at higher risk with a rate of an adverse 30-day outcome of 14.2 (95% CI, 8.6-21.5) % while 802 (72.2%) patients were (re-)classified

as intermediate-low risk (2.2; 95% CI, 1.3-3.5 % adverse outcomes) and 182 (16.4%) patients identified as low-risk (0; 95% CI, 0.0-2.0 % adverse outcomes).

Supplementary Discussion

Modification of the 2014 ESC algorithm using copeptin instead of imaging modalities

Limitations of imaging modalities for risk stratification of normotensive patients with acute PE include, among others, the limited availability of TTE twenty-four/seven especially in smaller hospitals and the ongoing debate on the reliability of MDCT for the assessment of RV dysfunction [6]. Therefore, and based on the good performance of copeptin with regard to the identification of higher risk PE patients, we replaced imaging modalities in the 2014 ESC algorithm by copeptin testing. Using this "modified" 2014 ESC algorithm, the identification of intermediate-high risk patients was comparable to the biomarker-based strategy and the "conventional" 2014 ESC algorithm (8.8% vs. 8.9% vs. 5.6% adverse outcomes of patients in the intermediate-high risk class, respectively).

Since copeptin testing is increasingly available especially in larger hospitals as a part of clinical routine (given the emerging role of copeptin in early diagnosis of acute myocardial infarction [7, 8, 9] and the incremental prognostic value of copeptin in PE [1, 10] and other diseases [11, 12]), measurement of copeptin (instead of RV imaging) may become a valuable alternative for risk assessment in normotensive PE, especially in hospitals not providing a twenty-four/seven TTE standby.

Supplementary References

(1) Hellenkamp K, Schwung J, Rossmann H, Kaeberich A, Wachter R, Hasenfuß G, Konstantinides S, Lankeit M. Risk stratification of normotensive pulmonary embolism: prognostic impact of copeptin. Eur Respir J 2015; 46: 1701-10.

(2) Becattini C, Agnelli G, Germini F, Vedovati MC. Computed tomography to assess risk of death in acute pulmonary embolism: a meta-analysis. Eur Respir J 2014; 43: 1678-90.

(3) Coutance G, Cauderlier E, Ehtisham J, Hamon M, Hamon M. The prognostic value of markers of right ventricular dysfunction in pulmonary embolism: a meta-analysis. Crit Care 2011; 15: R103.

(4) Morgenthaler NG, Struck J, Alonso C, Bergmann A. Assay for the measurement of copeptin, a stable peptide derived from the precursor of vasopressin. Clin Chem 2006; 52: 112-9.

(5) Instruction Manual B·R·A·H·M·S Copeptin us KRYPTOR assay.

(6) Jiménez D, Lobo JL, Monreal M, Moores L, Oribe M, Barrón M, Otero R, Nauffal D, Rabuñal R, Valle R, Navarro C, Rodríguez-Matute C, Alvarez C, Conget F, Uresandi F, Aujesky DA, Yusen RD; PROTECT investigators. Prognostic significance of multidetector CT in normotensive patients with pulmonary embolism: results of the protect study. Thorax 2014; 69: 109-15.

(7) Khan SQ, Dhillon OS, O'Brien RJ, Struck J, Quinn PA, Morgenthaler NG, Squire IB, Davies JE, Bergmann A, Ng LL. C-terminal provasopressin (copeptin) as a novel and prognostic marker in acute myocardial infarction: Leicester Acute Myocardial Infarction Peptide (LAMP) study. Circulation 2007; 115: 2103-10.

(8) Reichlin T, Hochholzer W, Stelzig C, Laule K, Freidank H, Morgenthaler NG, Bergmann A, Potocki M, Noveanu M, Breidthardt T, Christ A, Boldanova T, Merki R, Schaub N, Bingisser R, Christ M, Mueller C. Incremental value of copeptin for rapid rule out of acute myocardial infarction. J Am Coll Cardiol 2009; 54: 60-8.

(9) Maisel A, Mueller C, Neath SX, Christenson RH, Morgenthaler NG, McCord J, Nowak RM, Vilke G, Daniels LB, Hollander JE, Apple FS, Cannon C, Nagurney JT, Schreiber D, deFilippi C, Hogan C, Diercks DB, Stein JC, Headden G, Limkakeng AT Jr, Anand I, Wu AH, Papassotiriou J, Hartmann O, Ebmeyer S, Clopton P, Jaffe AS, Peacock WF. Copeptin helps in the early detection of patients with acute myocardial infarction: primary results of the CHOPIN trial (Copeptin Helps in the early detection Of Patients with acute myocardial Infarction). J Am Coll Cardiol 2013; 62: 150-60.

(10) Wyzgał A, Koć M, Pacho S, Bielecki M, Wawrzyniak R, Kostrubiec M, Ciurzyński M, Kurnicka K, Goliszek S, Paczyńska M, Palczewski P, Pruszczyk P. Plasma copeptin for short term risk stratification in acute pulmonary embolism. J Thromb Thrombolysis 2016; 41: 563-8.

(11) Nickel CH, Bingisser R, Morgenthaler NG. The role of copeptin as a diagnostic and prognostic biomarker for risk stratification in the emergency department. BMC Med 2012; 10: 7.

(12) Voors AA, von Haehling S, Anker SD, Hillege HL, Struck J, Hartmann O, Bergmann A, Squire I, van Veldhuisen DJ, Dickstein K; OPTIMAAL Investigators. C-terminal provasopressin (copeptin) is a strong prognostic marker in patients with heart failure after an acute myocardial infarction: results from the OPTIMAAL study. Eur Heart J 2009; 30: 1187-94.

Supplementary Figure legends

Figure 1s. Modified 2014 ESC algorithm

Copeptin was used instead imaging modalities to risk stratify patients according to the 2014 ESC algorithm which might constitute an attractive option in situation when echocardiography is not immediately available.

*According to the 2014 ESC algorithm, patients with a sPESI of 0 points and elevated hsTnT and / or NT-proBNP plasma concentrations were reclassified to the intermediate-low risk category.

A “positive” cardiac biomarker test refers to plasma concentrations of hsTnT ≥14 pg/ml and of NT-proBNP ≥600 pg/ml, respectively.

Supplementary Tables

Table 1s. Baseline characteristics and outcomes of patients included in the derivation cohort, validation cohort and pooled cohort

	Derivation cohort[1] (n=268)	**Validation cohort (n=843)**	**p-value**	**Pooled cohort (n=1111)**
Age (years)	71 [53-77]	70 [53-79], n=841	0.374	70 [53-78], n=1109
Sex (female)	140 (52.2%)	408 (48.4%)	0.293	548 (49.3%)
BMI (kg/m²)	27.8 [24.8-31.2], n=259	26.8 [24.0-30.0], n=620	**0.011**	27.0 [24.0-30.4], n=879
Comorbidities				
Active cancer	37 (13.8%)	126 (15.0%), n=842	0.692	163 (14.7%), n=1110
Chronic heart failure	42 (15.7%)	61 (7.2%), n=842	**<0.001**	103 (9.3%), n=1110
Chronic pulmonary disease	35 (13.1%)	89 (10.6%), n=842	0.266	124 (11.2%), n=1110
Renal insufficiency	73 (27.2%)	244 (29.2%), n=836	0.587	317 (28.7%), n=1104
Symptoms				
Chest pain	155 (58.1%), n=267	365 (43.3%), n=842	**<0.001**	520 (46.9%), n=1109
Dyspnoea	233 (87.3%), n=267	645 (76.6%), n=842	**<0.001**	878 (79.2%), n=1109
Syncope	32 (11.9%)	133 (15.8%), n=842	0.139	165 (14.9%), n=1110
Haemodynamic status at presentation				
Mild hypotension	19 (7.3%), n=262	34 (4.1%), n=830	**0.047**	53 (4.9%), n=1092
Tachycardia	80 (30.3%), n=264	309 (37.1%), n=834	**0.047**	389 (35.4%), n=1098
Hypoxia	62 (27.7%), n=224	135 (19.7%), n=687	**0.015**	197 (21.6%), n=911
RV dysfunction (on TTE)	77 (49.0%), n=157	195 (25.5%), n=766	**<0.001**	272 (29.5%), n=923
RV dysfunction (on TTE or MDCT)	132 (50.0%), n=264	267 (32.9%), n=811	**<0.001**	399 (37.1%), n=1075
Laboratory biomarkers				
hsTnT (pg/ml)	24.6 [8.6-57.9], n=266	19.3 [7.9-44.7]	0.072	20.3 [8.2-49.3], n=1109
hsTnT ≥14 pg/ml	166 (62.4%), n=266	504 (59.8%)	0.472	670 (60.4%), n=1109
NT-proBNP (pg/ml)	687 [122-2564], n=267	449 [115-2257]	0.070	479 [119-2348], n=1110
NT-proBNP ≥600 pg/ml	138 (51.7%), n=267	376 (44.6%)	**0.049**	514 (46.3%), n=1110
Copeptin (pmol/l)	13.7 [5.9-44.5]	9.6 [5.2-21.1]	**<0.001**	10.4 [5.4-24.0]
Copeptin ≥24 pmol/l	96 (35.8%)	181 (21.5%)	**<0.001**	277 (24.9%)

Risk classes				
sPESI ≥1 point(s)	168 (62.7%)	495 (58.7%)	0.254	663 (59.7%)
2014 ESC algorithm				
Low risk	47 (17.5%)	135 (16.0%)	0.570	182 (16.4%)
Intermediate-low risk	135 (50.4%)	460 (54.6%)	0.233	595 (53.6%)
Intermediate-high risk	86 (32.1%)	248 (29.4%)	0.402	334 (30.1%)
Outcome				
30-day adverse outcome	15 (5.6%)	21 (2.5%)	**0.017**	36 (3.2%)
30-day PE-related death	4 (1.5%)	12 (1.4%)	1.000	16 (1.4%)
30-day all-cause death	9 (3.4%)	36 (4.3%)	0.596	45 (4.1%)

Continuous variables are given as median [IQR] and categorical variables as absolute numbers (percentages); n refers to the number of patients with available data.

Abbreviations: BMI, body mass index; ESC, European Society of Cardiology; hsTnT, high-sensitivity troponin T; MDCT, multidetector computed tomography; NT-proBNP, N-terminal pro-brain natriuretic peptide; RV, right ventricular; sPESI, simplified Pulmonary Embolism Severity Index; TTE, transthoracic echocardiography; PE, pulmonary embolism.

Table 2s. Prognostic performance of copeptin in the derivation cohort, validation cohort and pooled cohort with regard to an adverse 30-day outcome

	Derivation cohort [1] (n=268)	**Validation cohort (n=843)**	**Pooled cohort (n=1111)**
Aura under the curve (AUC)	0.68 [0.52-0.83], p=0.020	0.70 [0.57-0.83], p=0.002	0.71 [0.61-0.81], p<0.001
Sensitivity (%)	73 [48-89]	62 [41-79]	67 [50-80]
Specificity (%)	66 [60-72]	80 [77-82]	76 [74-79]
Negative predictive value (NPV) (%)	98 [94-99]	99 [98-99]	99 [98-99]
Positive predictive value (PPV) (%)	11 [7-19]	7 [4-12]	9 [6-13]
Positive likelihood ratio (LR+)	2.2 [1.5-3.1]	3.0 [2.1-4.3]	2.8 [2.2-3.7]
Negative likelihood ratio (LR-)	0.4 [0.2-0.9]	0.5 [0.3-0.8]	0.4 [0.3-0.7]
Odds ratio (OR)	5.4 [1.7-17.6], p=0.005	6.3 [2.6-15.5], p<0.001	6.5 [3.2-13.2], p<0.001
Models for risk stratification including copeptin			
2014 ESC algorithm*: intermediate-high risk#	4.7 [1.5-14.1], p=0.006	5.0 [2.0-12.6], p=0.001	4.4 [2.3-8.5], p<0.001
2014 ESC algorithm plus copeptin: intermediate-high risk#	5.5 [1.9-16.0], p=0.002	11.1 [4.6-27.1], p<0.001	8.9 [4.5-17.5], p<0.001
Modified 2014 ESC algorithm: intermediate-high risk#	7.2 [2.4-21.9], p=0.001	7.4 [3.1-18.0], p<0.001	7.8 [3.9-15.6], p<0.001
Biomarker-based strategy: intermediate-high risk#	13.0 [4.0-42.7], p<0.001	7.0 [2.9-16.8], p<0.001	9.2 [4.6-18.5], p<0.001

* not including copeptin.

intermediate-high risk tested vs. intermediate-low and low risk.

Sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios and Odds ratio of copeptin ≥24 pmol/l and models for risk stratification with regard to an adverse 30-day outcome. 95% CIs are given in square brackets.

Abbreviations: ESC, European Society of Cardiology.